INDUCED AUTOTETRAPLOIDY IN RED CLOVER, T. *pratense* L., AND CRIMSON CLOVER T. *incarnatum* L.

BY

ROBERT J METZGER

B.S., University of Illinois, 1948
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SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN AGRONOMY
IN THE GRADUATE COLLEGE OF THE
UNIVERSITY OF ILLINOIS, 1953

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The present study was carried on for two and one half years, from January 1950, to June 1952. The purpose of the study was to observe the effects of induced polyploidy in red and crimson clover, to learn more about the place of this technique in a forage-crop breeding program.

Materials And Methods

Eleven hundred and sixty red clover seedlings, representing ten varieties, were treated with colchicine during the investigation. Eighty-three of the treated plants developed tetraploid sectors. Varieties represented by the induced tetraploids were Kenland, Rahn, Van Atta, Essex, Dollard, common mammoth, a southern anthracnose resistant selection of Dollard and a Purdue selection of red clover.

In the fall of 1951, 200 crimson clover seedlings of the Dixie variety were treated with colchicine. Seventeen of the resulting plants produced mixoploid sectors with varying chromosome numbers.

Percentage of aborted pollen and yield of seed were determined both for diploid and tetraploid sectors of colchicine treated plants (parental plants or C_0 generation), C_1 and C_2 generations. The term C_1 was defined to include all plants produced from seeds harvested from teraploid sectors of the C_0 plants. The term C_2 was defined to include all plants resulting from seeds harvested from the C_1 plants. Therefore, seeds resulting from self, hand-pollinations and insect pollinations were included in each generation.

Flower buds for the study of reduction division were fixed in Carnoy's fluid saturated with mercuric chloride. Preliminary results indicate the fixative will work equally as well

on tetraploid *T. incarnatum, T. repens, T. hybridum,* and *Glycine max.*

Most of the results reported in this investigation were from data collected in the greenhouse.

Experimental Results

Seed set and aborted pollen. In the parental generation (C_0) an average of 27.5 per cent of the hand-pollinated flowers set seed and 23.9 per cent of the pollen produced was shriveled. Differences between plants in seed yield were not statistically significant, but differences between plants for shriveled pollen were significant.

In the C_1, represented by 18 plants, 40.0 per cent of the hand-pollinated flowers in paired heads produced seed, and 15.2 per cent of their pollen was shriveled. Differences between plants both in seed set and aborted pollen were significant.

In the C_2, represented by forty plants, seed was harvested from 32.5 per cent of the hand pollinated flowers. An average of 21.9 per cent of their pollen was shriveled. Significant differences were found between plants both for seed set and aborted pollen.

A significant negative correlation was found between seed set and pollen abortion in the parental generation. However, this correlation was meaningless, since the differences between plants in seed set were not significant. The value found probably was due to chance and may have resulted from the combination of the small number of plants investigated, and an extreme deviation from the average in the performance of one of the plants. The correlation between seed set and pollen abortion was not significant in either the C_1 or C_2 generation.

Self-fertility. Self-fertile plants were observed in all three generations studied. The mode of inheritance of self-fertility was not studied, but preliminary observations indicated that some type of interaction of the sterility alleles was involved.

Effects of inbreeding on plant vigor. Limited data indicated there was very little if any decrease in vigor of first generation tetraploid inbreds grown in the greenhouse.

Significance of the variations observed in somatic chromosome numbers. One aneuploid, five triploids and three trisomics were observed for variations in morphological characteristics. Many of the extreme variations in size and shape of leaflets, reduced pubescence, high percentage of aborted pollen, and number of leaflets were associated with irregularities in chromosome numbers. However, in some cases normal tetraploids had fused flowers, a high percentage of aborted pollen, reduced pubescence and extra leaflets.

Meiosis in red clover. Reduction division is normal in diploid red clover. In contrast, many irregularities were observed during metaphase I and succeeding stages in tetraploid red clover. Unfortunately, stages prior to metaphase I were not found in diploid material and were observed infrequently in tetraploid material. The average number of quadrivalents observed during metaphase I in tetraploid red clover probably was too low to suggest any relationship between them and fertility.

Mixoploidy in crimson clover. Limited data indicated that approximately 21.1 per cent of the tripped flowers on mixoploid heads yielded seed and 23.2 per cent of the pollen they produced was shriveled. There was considerable variation in seed set and pollen abotrion depending on the degree of mixoploidy. Limited data indicated that irregularities in chromosome pairing were common at metaphase I in autotetraploid crimson clover. Meiosis was not investigated in diploid crimson clover.

Five tetraploid S_1 crimson clover plants produced flowers of which 23.0 per cent set seed and 21.8 per cent of their pollen was shriveled. Two of these plants were more vigorous than diploid plants, indcating that selected tetraploids may be of some value in a breeding program.

Interspecific crosses. A total of 22 crosses were attempted

between seven species of *Trifolium.* These were: diploid and tetraploid *T. pratense,* diploid and tetraploid *T. incarnatum,* tetraploid *T. hybridum,* tetraploid *T. repens* (ladino), *T. longipse, T. pannonimum* and *T. medium.* Self-sterile plants were used as the female parents, and in each kind of cross flowers were pollinated with a composite of pollen collected from the species used as the male parent.

Ninety seeds were harvested from 9648 hand-pollinated flowers on 84 plants. Seeds from crosses between autotetraploid red clover and autotetraploid crimson clover either were found to be selfs are failed to germinate. No viable hybrid seeds were produced.

Conclusions

Evidence presented in this investigation clearly indicates that new induced autotetraploids of red clover and crimson clover are of no immediate agronomic value. However, differences observed between plants in seed set, pollen abortion and many other characters suggests it may be possible to select for desirable agronomic characteristics.

VITA

The author was born on a farm near Cerro Gordo, Illinois, on April 11, 1925. He attended rural elementary schools there, and in 1943, was graduated from the Cerro Gordo Township High School.

He matriculated at the University of Illinois that same year, but interrupted his college training to enter the armed forces in February 1944. After being seperated from the armed forces in November 1945, he returned to the University of Illinois and received his B. S. degree in General Agriculture with a major in Agronomy in 1948. From November 1948, until August 1949, he was a graduate assistant at the University of Illinois and received his M. S. degree in Agronomy. From September 1949, until September 1951, he was garduaate assistant at the same school while studying toward a Ph.D. in Agronomy. In September 1951, he was appointed fellow in Agronomy an in January 1953, he was appointed Associate Agronomist at Clemson College, South Carolina.

The author is a member of Alpha Zeta.

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